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CLEANUP AND CONFIRMATION OF IDENTITY OF PESTICIDE RESIDUES BY THIN-LAYER CHROMATOGRAPHY PART I - SOIL, WATER, AND SEDIMENT

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An environmental monitoring program was started by Plant Pest Control Division in 1964 to determine the impact of agricultural pesticides on farmland (DIAP). This program requires pesticide residue analyses of hundreds of samples of soil, water, sediment, biologicals, and crops at the National Monitoring Laboratory, Gulfport, Miss.

Soil, water and sediment samples are analyzed by gas liquid chromatography (GLC). Confirmation of GLC results are required for many samples; however, valid results are obtained in many instances without cleanup. Existing methods employ column chromatography for separation and cleanup (2).[4] This method is tedious and time consuming. We have found that thin-layer chromatography (TLC) or a combination of TLC and other analytical methods gives best results for cleanup and confirmation (6, p. 150; 12; 15). When cleanup is required, a rapid, simple method is necessary because of the large volume of samples involved.

The method described herein for cleanup and confirmation of identity is rapid, both qualitatively and quantitatively, for many pesticides, and further cleanup is usually not required. The method has a sensitivity of approximately 0.1 to 1.0 µg.

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4/ Underscored figures in parentheses refer to Literature Cited at end of report.

APPARATUS AND EQUIPMENT[5/]

Thin-layer chromatography apparatus by Stahl (13)--Brinkmann Instruments, Inc., Great Neck, N. J.

Round developing tanks, 2-3/8 x 9 inches, Brinkmann Instruments, Inc.

Gas chromatograph, with electron affinity detector, F & M, Model 810.

Ultraviolet light source, General Electric 15 w. germicidal lamp, Model G15T8 in swing-arm desk lamp.

One-half-gallon fruit jars with tops.

U.S. Stoneware ball mill rollers.

Five-gallon bottles.

Kuderna-Danish 500-ml. evaporative concentrators with 10-ml. graduated receiver ampules.

Bausch and Lomb spectronic 20 colorimeter with constant voltage transformer.

Extraction tumbler (capable of rotating eight $\frac{1}{2}$-gallon jars concentrically).

Snyder distilling columns, 3 bulb, with 24/40 ground glass joints.

Centrifuge tubes, 13 ml. graduated to 0.1 ml., with ST 13 glass stoppers.

Constant temperature water bath with rack, to hold eight test tubes.

Erlenmeyer 250-ml. flasks, with 24/40 ground glass joints.

Erlenmeyer 250-ml. flasks, with wide mouth.

Disposable capillary pipettes, Kensington Scientific Corp.

Separatory funnels, 250-ml. with ST 22 joints.

Polaroid camera model 180, and Copymaker model 208.

Hair dryer, with stand and hot and cold air controls.

MATERIALS

Aluminum oxide G and silica gel G, Brinkmann Instruments, Inc.

Solvents, C. P., redistilled over sodium metal.

5/ The use of trade names and sources of supply is for identification purposes only and does not constitute endorsement by the U.S. Dept. of Agriculture.

Pesticides, reference standards in table 1 were dissolved in pentane to contain 10 ug./ml., City Chemical Corp., New York, N. Y.

Nuchar-Attaclay mixture, Wilkins Instrument and Research, Inc.

Test dyes, Azobenzene (Fisher No. 704) 25 mg./ml. benzene.

Brinkmann test dye mixture.

Iodine, U.S.P.

Fluorescein, reagent 0.2 percent w/v fluorescein in 95 percent ethanol.

Silver nitrate reagent, dissolve 0.8 g. silver nitrate in 5 ml. distilled water; add 10 ml. ethylene glycol monobutyl ether, dilute to 200 ml. with acetone (analytical grade), and mix. Add 1 to 5 drops of 3 percent hydrogen peroxide. Store in brown bottle when not in use (10).

METHODS

Preparation of Plates

Silica gel layers 250 ul. thick were applied on glass plates 200 x 50 mm., according to Stahl (13). After drying 15 minutes at ambient temperature, the plates were activated for 30 minutes in an oven at 105° to 120° C., then allowed to cool in a desiccator.

The 200-mm. x 200-mm. plates were coated in the same way, with one innovation: 1 ml. of 0.2 percent fluorescein in ethanol was added to the slurry prior to application (9).

Extraction

Soil and Sediment

Three hundred grams of screened soil, with allowance for moisture content, was extracted by concentric rotation for 4 hours in 600 ml. of 3:1 mixture of hexane-isopropyl alcohol in ½-gallon fruit jars.

Sediment is extracted in the same way, except that 150 g. of anhydrous Na_2SO_4 is added to the jar.

Extract equivalent to a 50-g. portion of soil or sediment was filtered into a 250-ml. separatory funnel and washed twice with 100 ml. of distilled H_2O. Several milliliters of a saturated solution of $NaHCO_3$ were used to break any emulsions occurring. After drying over Na_2SO_4, the remaining hexane solution was quantitatively transferred with washing to a 100-ml. brown bottle with stopper. The sample was then analyzed by GLC.

Water

Five gallons of water were weighed and decanted into an extraction bottle, leaving any sediment in the sample bottle. The extraction bottle was weighed to get the corrected weight of the water. One liter of 3:1 redistilled pentane-ether solution was added, and the bottle closed and rotated 20 minutes at 30 r.p.m. on ball mill rollers.

A sufficient quantity of water was then added to the bottle to displace the upper solvent layer into a graduate by means of a delivery tube. This solvent was dried over anhydrous Na_2SO_4 and concentrated on a water bath to 100 ml. for GLC.

Procedure

If further cleanup is required, the sample is processed in the following manner:

The sample containing excessive amounts of interfering material, as indicated by high color or organic matter present prior to TLC, was transferred to a 250-ml. glass-stoppered Erlenmeyer flask, and 0.1 g. of Nuchar-Attaclay mixture added. After shaking for 1 minute, the solution was filtered into a Kuderna-Danish evaporative concentrator and heated on the water bath until about 5 ml. of solution remained. This was transferred to a 15-ml. centrifuge tube and further concentrated to 0.1 ml. in a 40° C. water bath by a gentle stream of dry air.

The concentrate was then spotted with a capillary pipette as a band about 1.0 to 2.0 cm. long, 2.5 cm. from the bottom, on a 200-mm. x 500 mm. plate (4, p.53). Approximately 0.1 ml. of pentane was added to the centrifuge tube as a wash and spotted also. Since it is necessary to keep the band as small as possible, all the solvent was evaporated after each spotting in a current of warm air from a hair dryer. When the band had dried, the plate was developed in two solvents: benzene first, then pentane or hexane. A sufficient quantity of each of the two solvents was measured separately into each of two circular developing tanks to cover the tank bottoms 1 cm. in depth (about 25 ml.), and the tanks equilibrated for approximately 30 minutes at ambient temperature. A sheet of blotting paper was also placed in each. The plate was placed consecutively in the two solvents that were allowed to ascend on the plate 5 and 10 cm., respectively, from the origin. Between each development, the plate was set aside to dry. Total development time was approximately 20 minutes. When the last solvent had evaporated, the plate was divided into two sections: an upper one, from 5.0 to 10.0 cm., and a lower one, from 0.5 to 5.0 cm. (5, p.33; 15) (Fig. 1). The two sections were scraped off, collected separately into glass tubes by suction, and eluted with redistilled benzene, for GLC, colorimetric or infrared analysis (3).

Qualitative Confirmation

An 0.1-ml. aliquot containing the equivalent of 5 g. of the original substrate was spotted on a 200-mm. x 200-mm. fluorescein impregnated plate, 2.5 cm. from the lower left edge (Fig. 2). Standard insecticides were spotted in the lower

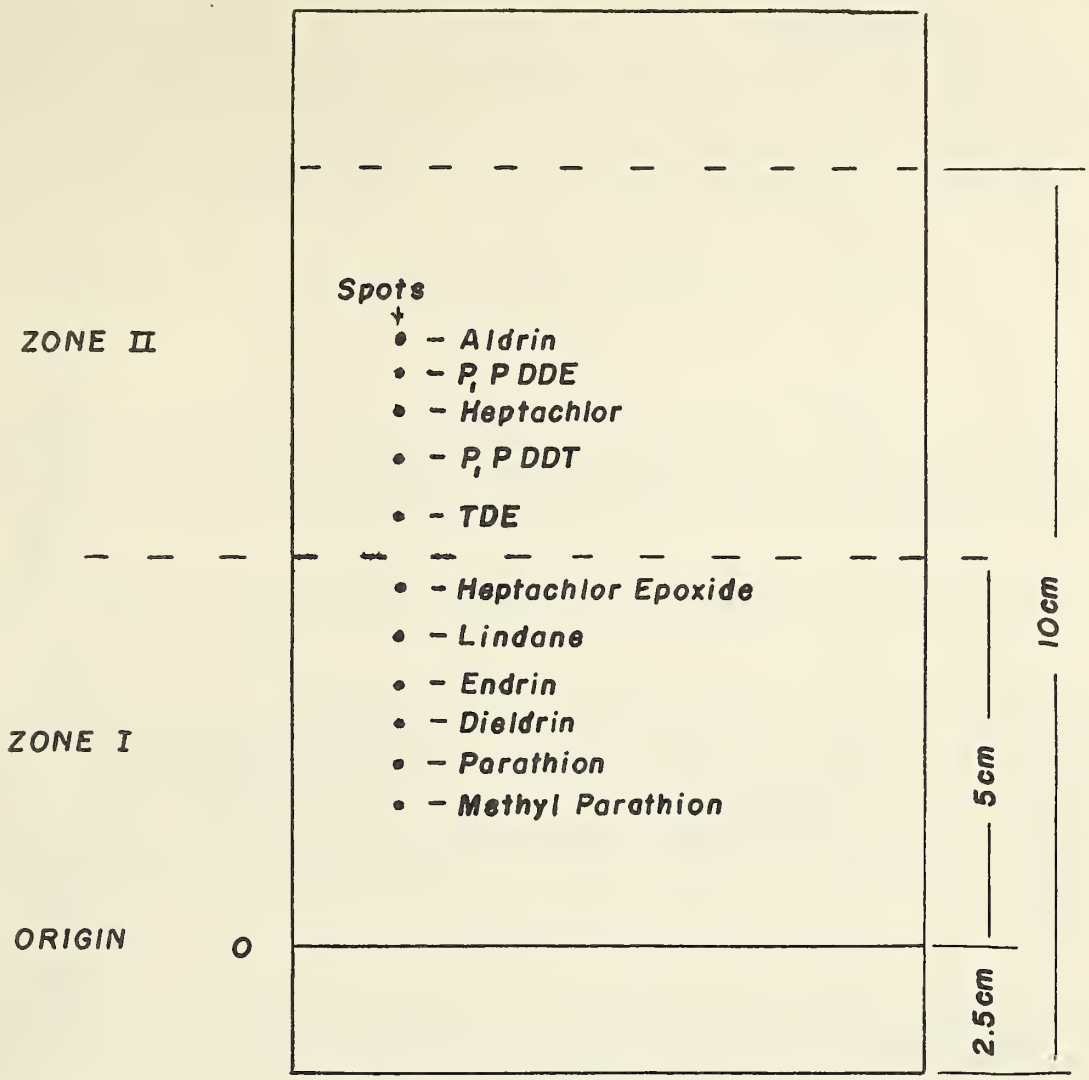

Figure 1. *Separation of insecticides into two zones by TLC stepwise development.*

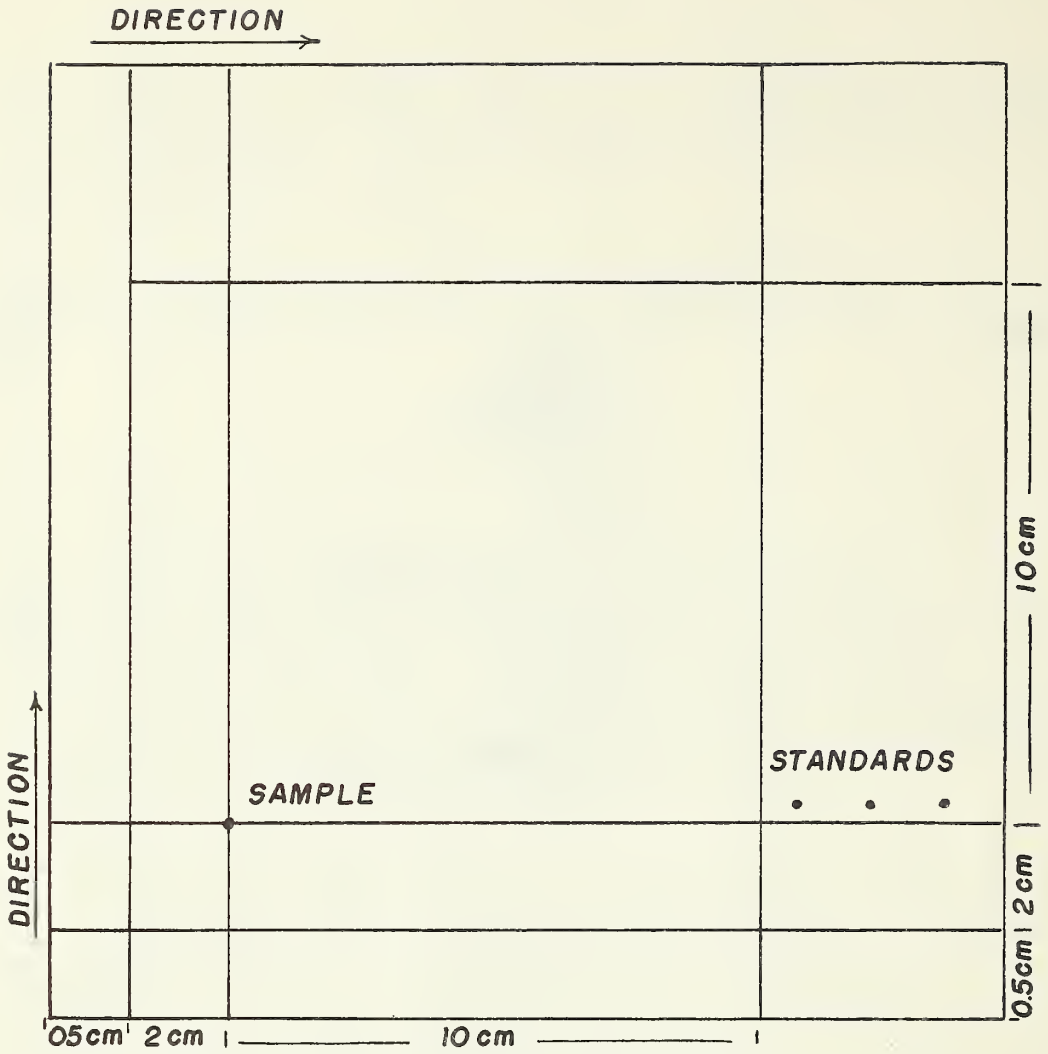

Figure 2. Two-diminsional TLC development

right side of the plate, 11 cm. or more to the right of the sample spot. The plate was developed in 9:1 pentane-ether to a height of 10 cm., and the solvent allowed to evaporate. The plate was rotated 90° to the left (Fig. 2) and again developed in the same solvent to the 10 cm. mark. When the last solvent had evaporated, the plate was visualized by treatment with the iodine, fluorescein, and silver nitrate reagents in that order, and placed under ultraviolet light (16). The plate was then ready for evaluation.

RESULTS AND DISCUSSION

Coatings

Silica gel G was best suited for separating chlorinated hydrocarbons and organophosphates and many other pesticides encountered, because it gave the greatest range of Rf values.6/ The thickness of the coating was increased to 500 ul. if the samples contained too much interference resulting in overloading of the plates.

In cases where endrin and dieldrin need to be separated, aluminum oxide G was the best medium (5, p.29).

When TLC was used as a cleanup for GLC, it was necessary to use redistilled ether to remove impurities from the coatings. Prior to spotting the samples, the ether was allowed to ascend the length of the plate in a developing tank.

The activity of each set of plates was checked each day by spotting 2 ul. of test dye on a plate and developing it in the same way as a sample.

Solvent Systems

In the separation and cleanup of samples for GLC, two solvents were used stepwise, because it was necessary to separate the two classes of insecticide: organophosphates and chlorinated hydrocarbons (4, p.72). Benzene was used first to move the organophosphates away from the origin, then pentane or hexane was used to move most of the chlorinated insecticides up the plate and leave the organophosphates in the lower part (14). Hexane, or pentane, gave the best overall separation of insecticides. The more polar solvents tried--$CHCl_3$, C_6H_6, and CCl_4--carried all the chlorinated insecticides together into the top half of the plate (16).

Development

Just as the two-step development was best for separating two different classes of insecticides, so two-dimensional development was found best for removing insecticides from interference (4, p.74: 6, p. 147). When the plate is developed in two different dimensions, the interferences are left in one direction whereas the insecticides are carried in another direction.

6/ $Rf = \frac{\text{distance traveled by a given substance}}{\text{distance traveled by solvent front}}$

Recoveries

Colorimetric procedures subsequent to TLC cleanup did not give us as good recovery as GLC. For example, the phenyl azide method (8) for aldrin and dieldrin gave only about 72 percent recovery (Fig. 3), as compared with 97 percent recovery for aldrin and dieldrin by GLC. This latter value is in line with those reported by Morley and Chiba (11). Recovery of all pesticides in table 1 by the TLC-GLC procedure was at least 90 percent, or higher.

Table 1.--Rf values for various solvent systems

Pesticides	Benzene-hexane 1/2/	Benzene-pentane 1/2/	(9 + 1) Pentane-ether 2/	Hexane 3/
Aldrin	0.64	0.70	0.85	0.87
Heptachlor	.60	.64	.80	.83
Heptachlor epoxide	.43	.45	.51	.35
Lindane	.45	.47	.43	.47
Dieldrin	.34	.34	.35	.30
Endrin	.35	.37	.38	.35
TDE	.50	.53	.61	.59
p,p-DDT	.56	.62	.73	.76
o,p-DDT	.56	.62	.73	.76
p,p-DDE	.64	.65	.82	.85
Methyl parathion	.25	.25	.15	0
Parathion	.30	.27	.35	0
Strobane	.40-.65	.40-.68	.40-.85	.40-.65
Toxaphene	.40-.65	.40-.68	.50-.80	.40-.60

1/ Two-step development.

2/ Developed on silica gel.

3/ Developed on Al_2O_3.

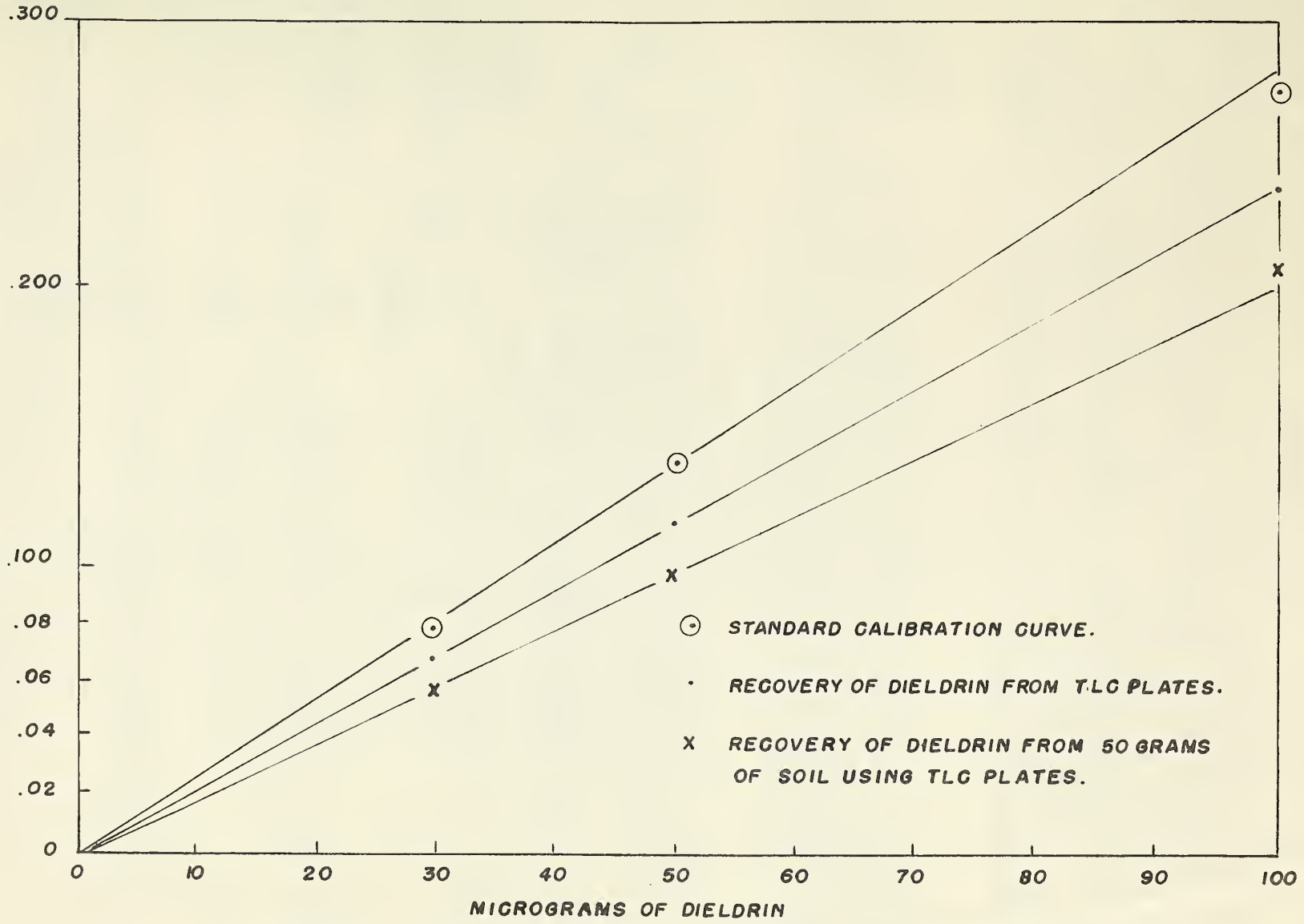

FIGURE 3. - STANDARD DIELDRIN AND DIELDRIN RECOVERY CURVES

Interferences

Most of the soils used in this work were low in organic matter and did not cause excessive interference. In many sediment and water samples, sulfur was found. This gave a large yellowish spot in the presence of fluorescein and $AgNO_3$. It also obscured results in many cases, as it has an Rf[7/] in the vicinity of p,p-DDE, heptachlor, and aldrin. In GLC, it has a retention time the same as lindane or heptachlor, depending on which allotropic form is present. Strobane and toxaphene interfere with most of the chlorinated hydrocarbons in the top half of the TLC plate. Since strobane and toxaphene are mixtures, they cannot be analyzed by GLC. It is necessary to determine them colorimetrically by taking the eluate off the thin-layer plate (7).

Visualization

The combination of iodine, fluorescein, and silver nitrate gave best results in bringing out all the spots containing chlorinated hydrocarbons, as most of these reacted with at least one of the three reagents. Other spray reagents giving good results are listed in table 2. Iodine crystals were more convenient to use than the bromine solution employed by Walker and Beroza and gave results that were entirely satisfactory (1, 16).

Recording of Data

The plates were photographed in a darkroom in ultraviolet light or artificial light with a Polaroid camera and copymaker as soon as possible after visualization, and the picture kept with the sample report, if necessary.

Table 2. Some spray reagents for chlorinated pesticides (4, p.88)

Reagent	Type plate	Color in daylight	Remarks
Iodine	Silica gel, Al_2O_3	Brown spots on yellow background	Expose to I_2 crystals for at least 2 min. until some spots appear.
Iodine-benzidene	Silica gel, Al_2O_3	Brown spots on blue background	Expose to I_2 crystals and then spray with benzidene.
Silver nitrate-fluorescein	Silica gel	White to yellow spots on rose background	Expose to ultraviolet light for 7 to 10 min.
N-N dimethyl p-phenylene diamine-2HCl Na O Me	Al_2O_3	Green violet spots	Spray with compound, moisten with water, expose to ultraviolet light without filter

7/ See footnote 6.

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